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SOME RESEARCH NEEDS TO SUPPORT MUTAGENIC RISK ASSESSMENTS FROM WHOLE MAMMAL STUDIES

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16. ABSTRACT

This paper presents an approach to use DNA binding data to obtain information as to the possible shape of the dose response curve at low chemical exposure levels in whole mammals. Such information will be useful for both mutagenic and carcinogenic risk estimations in which high to low dose extrapolations are necessary.

The paper also discusses the uses of genetic damage tests that can be performed in both humans and experimental mammals and how these tests may be useful in estimating mutagenic risks.

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1. INTRODUCTION/BACKGROUND

The primary goal of regulatory agencies concerned with human health is to ensure that exposure to chemical substances does not present unreasonable risks. This involves assessing the potential of chemical substances to cause toxic effects and weighing these effects against cost and benefit considerations. If the toxicity and exposure data allow, attempts are made to estimate health damage to the human population. For most environmental chemicals, human data are unavailable. On those occasions when chemical exposure can be associated with a toxicological response in humans, many people have already been exposed and irreparable damage may have occurred. Examples of such events include the kepone incident, neurological disorders; the dibromochloropropane incident, sterility; and the vinyl chloride incident, liver angiosarcomas. Therefore, it is imperative that animal toxicity studies be conducted to assess the likely human health risk before such effects are observed.

The U.S. Environmental Protection Agency (EPA) is faced with the task of making health risk assessment judgments on the thousands of chemicals presently in the environment as well as on new industrial chemicals that are entering the environment in increasing numbers (Table I). Unfortunately, for many of those chemicals little if any animal toxicological data exist. For example, animal and/or *in vitro* data were available for only 208 out of 400 organic chemicals identified at Love Canal[1]. Furthermore, in most cases such data are inadequate to make a judgment as to the likely toxicological effects in humans.

It is important that the federal government, private industry, and the scientific community at large integrate current methodology to put risk

assessments on a more sound scientific basis. We have definitive proof that exposure to certain chemicals induces cancer in the human population[2], but no proof that chemicals induce heritable mutations that can lead to serious genetic disorders. However, there are strong suggestive indications that they do. First, there already exists a high incidence of diseases in humans which are caused by heritable mutations[3]. Second, within the last decade specific locus data have shown that chemicals can cause heritable mutations in mammals[4], and, more recently, ethyl nitrosourea has been shown to cause serious dominant skeletal disease in mice[5]. Third, the main germinal target molecule is DNA in both humans and mice. In light of these observations, it is reasonable to assume that exposure to chemicals shown to be mutagenic in mouse test systems would also have the potential to be mutagenic in humans and could lead to serious debilitating heritable genetic disease.

The EPA currently operates under seven statutes, which not only provide the basis for regulatory action, but also provide the Agency with the authority to perform health risk assessments. The wording in these statutes provides for the use of mutagenicity data on environmental pollutants to assess heritable genetic disease. For example, the Toxic Substances Control Act[6] includes mutagenesis along with teratogenesis and carcinogenesis as toxicological endpoints of concern and for which testing standards are to be developed.

As a first step towards fulfilling these mandates, the EPA proposed general guidelines for mutagenicity assessment in which test systems were described that would be acceptable for determining the "intrinsic" mutagenicity of a chemical, its ability to reach the germ cells, and possible quantitative approaches to estimate human heritable disease incidences. These

guidelines, published in the Federal Register[7] in 1980, constituted the first attempt by any regulatory agency to consider mutagenicity as a distinct toxicological endpoint rather than merely using mutagenicity as an indicator of carcinogenicity.

The weight-of-evidence approach outlined in the guidelines (Table II) is composed of two parts. The first part is a qualitative assessment of whether or not a chemical substance causes mutations and whether or not it reaches germinal tissue in an active form. If the data allow, a quantitative assessment of the risk may be performed, which can be based on whole mouse heritable mutagenicity data.

Public comments were solicited on the proposed mutagenicity guidelines and the major concern was that the guidelines were premature. This was based, first, on the lack of human data demonstrating that exposure to chemical agents increases the incidence of heritable genetic diseases and, second, on the lack of sufficient experimental mammalian studies to make quantitative mutagenicity assessments. These criticisms are valid. With respect to the first, it seems unlikely that in the near future we will be able to obtain sufficient human data to determine genetic risk resulting from exposure to environmental chemicals. However, as discussed previously there is indirect evidence indicating that exposure to chemical mutagens has the potential to increase the incidence of genetic disease in the population. Because of the potential of environmental chemicals to cause heritable genetic diseases, current regulatory statutes provide for use of mutagenicity data to protect human health. The remainder of this report will provide some thoughts on how the use of whole mammal mutagenicity tests may be enhanced to better estimate the possible risk of increasing heritable genetic diseases in the human population.

2. MOUSE BIOASSAYS

Whole mammal mutagenicity tests can demonstrate that a chemical has mutagenic activity, reaches germinal tissues, and causes mutations that are transmitted to the next generation, i.e., are heritable. However, one has to realize that, because of interspecies differences, any mammalian system can only approximate the various steps between exposure (inhalation, ingestion, or dermal absorption) and the fixation of mutations in human germinal cells. If one assumes that these steps in animal models, such as the mouse specific locus tests and mouse dominant skeletal and cataract tests, are representative of events occurring in humans, then extrapolations can be made from animal results to predict the occurrence of similar events in humans. This can be accomplished by using the "relative mutation risk approach," which involves mutiplying the ratio of induced mutations per locus caused by chemical exposure to the spontaneous rate of the test system by the spontaneous genetic disease incidences in the current human population. When animal tests score for actual genetic disease caused by chemical exposure, such as in the test for serious skeletal defects or cataracts, then a more direct method can be used to predict heritable disease in man. Details of both the relative risk and direct methods have been extensively described in the National Academy of Sciences' report on the "Effects on Populations of Exposure to Low Levels of Ionizing Radiation"[8]. Depending on the risk estimation method employed, different assumptions regarding spontaneous and induced mutation frequencies and disease incidences in both mice and humans are made. If results of the two methods are similar, then more confidence could be placed in their use. By employing the relative mutation risk approach using the mouse visible specific locus test, it was estimated that exposure to 1 rem of ionizing radiation would increase the incidence of

serious dominant heritable disorders per million first-generation live births between 8 and 40, whereas, with the direct method using the mouse dominant skeletal test, a range of 5 to 65 was estimated. It appears that both approaches are in agreement for ionizing radiation. Unfortunately, comparisons between these two approaches are not as rigorous for chemical mutagenesis since only 25 chemicals have been tested in the mouse specific locus test[4], and only one chemical, ethyl nitrosourea, has been tested in the dominant skeletal assay thus allowing application of the direct method. There is a need to increase the data base on environmental chemical mutagens in whole mouse bioassays, and also a need to compare both risk estimation methods for different classes of mutagenic chemical substances.

The whole mouse test systems provide the best data for use in estimation of genetic risk. However, there are some drawbacks to the systems as they are currently employed. It should be noted that the mouse specific locus test was not designed for risk estimation purposes. Humans are most likely to be exposed to environmental toxicants at "relatively" low levels and for long periods of time. In the whole mammalian bioassays used to measure mutagenicity, very high and often unrealistic exposures are given. The shape of the exposure/response curve at actual environmental levels cannot be determined from these studies. This means that, if the animal data are to be used for risk assessment purposes, extrapolations of mutagenicity risk from high to low exposure must be made. The consequences of this cannot be determined but serious under- or over-estimates of risk could be made. It would be very costly and impractical to increase the sensitivity of the whole mammal bioassays by greatly increasing the number of animals tested and administering low levels of the test compounds to determine the shape of the

exposure/mutagenic effect curve at low exposure levels. Because of this, other types of data, which are more readily obtainable, are needed to provide insight about the shape of the exposure response curve critical for the estimation of mutagenic risk in humans. One way this might be accomplished is to expose animals to radioactively-labeled mutagens both at high levels where mutations are being scored and at the environmental levels to which humans are exposed to obtain a relationship between external exposure and the amount of the substance reaching the gonads. Since gene mutations appear to be directly related to the amount of mutagen bound to DNA[9], then this curve could be used as a surrogate for the exposure/mutagenic effect curve at low exposure levels where mutations cannot be scored. Thus, some of the uncertainties and criticisms associated with extrapolation from high doses, where the effect of mutations are observed, to low doses, where effects cannot be observed, can be eliminated. Therefore, from the standpoint of risk assessment, any future mutagenicity testing of environmental chemicals should utilize approaches enabling an estimation of the shape of the lower end of the exposure/response curve.

3. USE OF GENETIC TOXICITY TESTS WITH WHOLE MAMMAL BIOASSAYS

There are several biological indicators of genetic toxicity available that can be measured in both humans and test animals (Table III). They can provide evidence that a mutagen enters the body and has the capacity to cause genetic damage in either somatic or germinal tissues. Similar genetic responses obtained in both mice and humans indicate that the chemical's pharmacokinetic properties in both organisms result in the same effect. Such supportive interspecies data provide more confidence that use of an animal model is a reasonable approach to estimate human mutagenic risk. As combinations of these tests are utilized and supportive interspecies data become more developed, it is hoped that some of the biological indicators will be sensitive and predictive enough to be used as environmental monitors for humans exposed to potential mutagens. Such tests could also play an important role for quantifying human mutagenic risk in the future. For example, the whole mammal bioassay could be performed in such a manner that indicators of genetic damage are measured in the exposed parents and heritable mutations are scored in the offspring. The measured induced mutation frequency or increased genetic disease incidence observed in the experimental animals could be expressed in terms of incidences of genetic damage [e.g., RBC variants, HGPRT mutations, LDH_X "mutations," sister chromatid exchange (Table III)], rather than exposure. When such data are available, an increase in one (or more) of the biological indicators observed in humans can potentially be used to estimate human genetic risk based on the measured relationship between the same biological indicator in mice and mouse mutation frequency. Because the occurrence of the indicator endpoints represents an integrated measurement of

all events between external exposure and damage to the cells scored, many assumptions about absorption and metabolism between the species are avoided in this approach. This concept is illustrated in Figure 1.

4. SUMMARY

In the last two decades humans have become critically aware of the threat of cancer as a consequence of exposure to certain environmental agents. More recently, people are becoming concerned about the potential adverse effects of environmental chemicals capable of causing serious genetic diseases. This awareness and concern comes primarily from findings in "short-term" mutagenicity screening tests, which indicate that many environmental chemicals have the potential to cause germinal mutations in humans. Regulatory agencies with legislative mandates to protect human health are often faced with making decisions based on toxicological data that are inadequate for estimation of the severity of the genetic risk, even in qualitative terms. In this paper we suggest that two additional types of measurements be made parallel with whole mouse mutagenicity tests when possible. First, the shape of the germinal DNA dose/external exposure curve should be determined for all chemicals being tested. These data would aid in extrapolating the likely genetic risk from the high experimental to the low environmental levels of exposure. Second, biological tests that indicate genetic damage should be performed in both experimental animals and humans to eliminate some of the assumptions used in interspecies extrapolations. These approaches have the potential to improve our mutagenicity risk assessment capabilities, but well-designed and carefully coordinated experimentation is needed. This will require expert input from the federal government, universities, private industry, and the scientific community at large. Hopefully, recent scientific advances described in other papers in this symposium will stimulate the necessary research support needed to improve current methodologies for assessing the mutagenic risk of chemicals.

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TABLE I. STATUS OF CHEMICALS IN THE ENVIRONMENT

60,000 CHEMICALS PRESENTLY IN COMMERCIAL PRODUCTION

365 NEW CHEMICALS INTRODUCED IN 1980*

659 NEW CHEMICALS INTRODUCED IN 1981*

164 NEW CHEMICALS INTRODUCED IN JANUARY AND FEBRUARY OF 1982*

*WHILE THESE CHEMICALS WERE PROPOSED FOR MANUFACTURING, EXPERIENCE INDICATES THAT LESS THAN HALF ARE ACTUALLY INTRODUCED INTO COMMERCE.

TABLE II. WEIGHT OF EVIDENCE APPROACH

I. QUALITATIVE ASSESSMENT

A. DOES THE CHEMICAL HAVE INTRINSIC MUTAGENICITY?

B. DOES EVIDENCE EXIST TO SHOW THAT THE CHEMICAL REACHES GONADAL TISSUE?

II. QUANTITATIVE ASSESSMENT

A. TO WHAT EXTENT ARE HERITABLE MUTATIONS INDUCED BY ENVIRONMENTAL EXPOSURES TO THE CHEMICAL?

B. WHAT FRACTIONS OF THESE INDUCED MUTATIONS RESULT IN GENETIC DISEASE?

TABLE III. SOME GENETIC TOXICITY TESTS THAT CAN BE USED IN BOTH HUMANS AND MICE

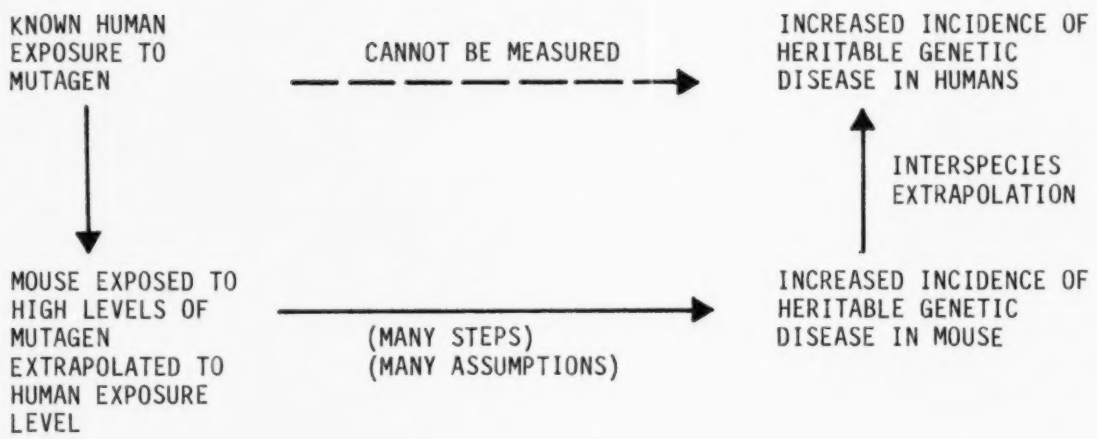
TESTS THAT MEASURE SOMATIC EFFECTS	TESTS THAT MEASURE GERMINAL EFFECTS
HGPRT MUTATIONS IN LEUCOCYTES	ALTERED SPERM COUNTS/ MORPHOLOGY/MOTILITY
RBC VARIANTS	LDH_X "MUTATIONS"
SISTER CHROMATID EXCHANGE	
CHROMOSOMAL ABERRATIONS	

FIGURE 1. A POTENTIAL USE FOR GENETIC TOXICITY TESTS IN QUANTITATIVE MUTAGENIC RISK ASSESSMENT.

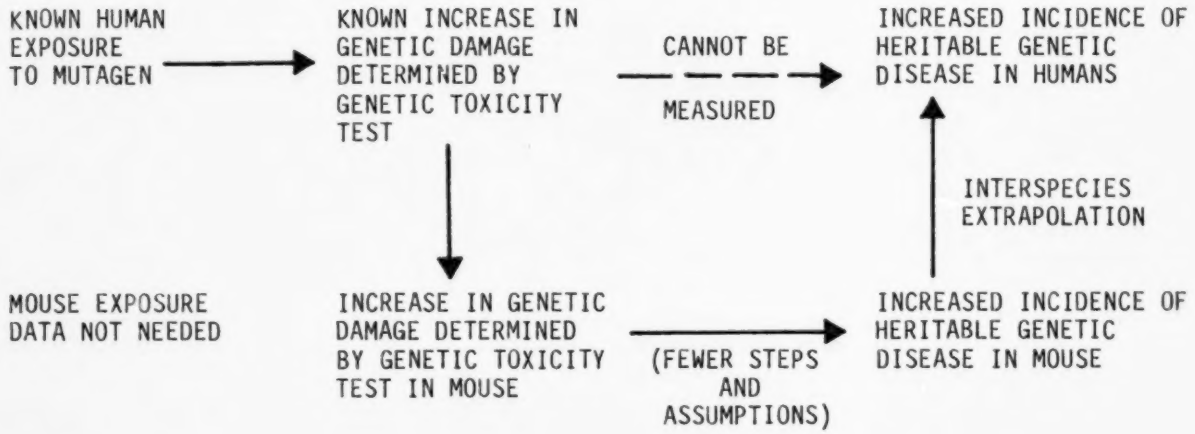
A. SHORT-TERM GENETIC TESTS ARE NOT USED.

B. SHORT-TERM GENETIC TESTS ARE USED.

A.


KNOWN HUMAN EXPOSURE TO MUTAGEN
CANNOT BE MEASURED
INCREASED INCIDENCE OF HERITABLE GENETIC DISEASE IN HUMANS
INTERSPECIES EXTRAPOLATION
MOUSE EXPOSED TO HIGH LEVELS OF MUTAGEN EXTRAPOLATED TO HUMAN EXPOSURE LEVEL
(MANY STEPS)
(MANY ASSUMPTIONS)
INCREASED INCIDENCE OF HERITABLE GENETIC DISEASE IN MOUSE

B.


KNOWN HUMAN EXPOSURE TO MUTAGEN
KNOWN INCREASE IN GENETIC DAMAGE DETERMINED BY GENETIC TOXICITY TEST
CANNOT BE MEASURED
INCREASED INCIDENCE OF HERITABLE GENETIC DISEASE IN HUMANS
INTERSPECIES EXTRAPOLATION
MOUSE EXPOSURE DATA NOT NEEDED
INCREASE IN GENETIC DAMAGE DETERMINED BY GENETIC TOXICITY TEST IN MOUSE
(FEWER STEPS AND ASSUMPTIONS)
INCREASED INCIDENCE OF HERITABLE GENETIC DISEASE IN MOUSE